

Amendments to the Claims

65. (Currently Amended) A method for detecting a target nucleic acid, the method comprising the steps of:

separating a sample in which a target nucleic acid is present in an amount less than about 20% relative to non-target nucleic acid in said sample, to form a plurality of assay samples;

amplifying said target nucleic acid in said assay samples;

hybridizing the amplification product to a first molecular beacon probe which hybridizes to the target nucleic acid and to a second molecular beacon probe which hybridizes to the non-target nucleic acid, wherein each of the first and the second molecular beacon probes comprises a photoluminescent dye and a quenching agent at opposite 5' and 3' ends, wherein the photoluminescent dye on the first and second molecular beacon probes are different, and wherein the first molecular beacon probe further comprises a ~~first stem and~~ a loop, wherein the stem ~~which~~ comprises about 4 base pairs having a sequence 5'-CACG-3', and ~~a first the loop structure which~~ a first the loop structure ~~which~~ comprises about 16 base pairs and has a T_m of about 50-51°C; and

detecting the first and the second molecular beacon probes hybridized to the target nucleic acid, thereby detecting the target nucleic acid.

66. (Currently Amended) A method for detecting a target nucleic acid, the method comprising the steps of:

providing a sample comprising X% of a target nucleic acid, wherein X is less than 100;

dividing the sample to produce a plurality of assay samples;

wherein the ratio of target to non-target nucleic acid in at least one of the samples is greater than X%;

amplifying the target nucleic acid to form an amplification product;

hybridizing the amplification product to a first molecular beacon probe which hybridizes to the target nucleic acid and a second molecular beacon probe which hybridizes to the non-target nucleic acid, wherein each of the first and the second molecular beacon probes comprises a photoluminescent dye and a quenching agent at opposite 5' and 3' ends, wherein the photoluminescent dye on the first and second molecular beacon probes are different, and wherein the first molecular beacon probe further comprises a ~~first stem~~ and a loop, wherein the ~~stem which~~ loop comprises about 4 base pairs having a sequence 5'-CACG-3', and a ~~first the loop structure which~~ loop comprises about 16 base pairs and has a T_m of about 50-51°C; and

detecting the first and second molecular beacon probes hybridized to the target nucleic acid, thereby detecting the target nucleic acid.

67. (Currently Amended) A method for detecting a target nucleic acid in a population of non-target nucleic acid contained in a sample, the method comprising:

dividing a heterogenous sample comprising target nucleic acid and non-target nucleic acid to form a plurality of assay samples, wherein the concentration of non-target nucleic acid is at least 5-fold that of target nucleic acid in the heterogeneous sample, and wherein at least one of the assay samples comprises a single molecule of the target nucleic acid molecule;

amplifying the single molecule of target nucleic acid to form an amplification product;

hybridizing the amplification product to a first molecular beacon probe which

hybridizes to the target nucleic acid and a second molecular beacon probe which hybridizes to the non-target nucleic acid, wherein each of the first and the second molecular beacon probes comprises a photoluminescent dye and a quenching agent at opposite 5' and 3' ends, wherein the photoluminescent dye on the first and second molecular beacon probes are different, and wherein the first molecular beacon probe further comprises a ~~first stem and a loop, wherein the stem which~~ comprises about 4 base pairs having a sequence 5'-CACG-3', and a ~~first the loop structure which~~ comprises about 16 base pairs and has a T_m of about 50-51°C; and

detecting the first and the second molecular beacon probes hybridized to the target nucleic acid, thereby detecting the target nucleic acid.

68-99. (Canceled)

100. (Currently Amended) The method of claim 65, 66, or ~~68~~ 67, wherein the second molecular beacon probe comprises a ~~second~~ stem and a ~~second~~ loop structure, wherein the ~~second~~ loop comprises about 19-20 base pairs and has a T_m of about 54-56°C, and wherein the ~~second~~ stem comprises about 4 base pairs having a sequence 5'-CACG-3'.

101. (Currently Amended) The method of claim 100, wherein the ~~second~~ loop of the second molecular beacon probe consists of 19-20 base pairs and has a T_m of 54-56°C, and wherein the ~~second~~ stem of the second molecular beacon probe consists of 4 base pairs having a sequence 5'-CACG-3'.

102-109. (Canceled)

110. (Currently Amended) A method for detecting a target nucleic acid, the method comprising the steps of:

separating a sample in which a target nucleic acid is present in an amount less than

about 20% relative to non-target nucleic acid in said sample, to form a plurality of assay samples;

amplifying said target nucleic acid in said assay samples;

hybridizing the amplification products to a first molecular beacon probe which hybridizes to the target nucleic acid and to a second molecular beacon probe which hybridizes to the non-target nucleic acid, wherein each of the first and the second molecular beacon probes comprises a photoluminescent dye and a quenching agent at opposite 5' and 3' ends, wherein the photoluminescent dye on the first and second molecular beacon probes are different, and wherein the second molecular beacon probe further comprises a ~~second~~ stem and a loop, wherein the stem ~~which~~ comprises about 4 base pairs having a sequence 5'-CACG-3', and ~~a second the loop structure which~~ comprises about 19-20 base pairs and has a T_m of about 54-56°C; and

detecting the first and the second molecular beacon probes hybridized to the target nucleic acid, thereby detecting the target nucleic acid.

111. (Currently Amended) A method for detecting a target nucleic acid, the method comprising the steps of:

providing a sample comprising X% of a target nucleic acid, wherein X is less than 100;

dividing the sample to produce a plurality of assay samples;

wherein the ratio of target to non-target nucleic acid in at least one of the samples is greater than X%;

amplifying the target nucleic acid to form an amplification product;

hybridizing the amplification products to a first molecular beacon probe which

hybridizes to the target nucleic acid and a second molecular beacon probe which hybridizes to the non-target nucleic acid, wherein each of the first and the second molecular probes comprises a photoluminescent dye and a quenching agent at opposite 5' and 3' ends, wherein the photoluminescent dye on the first and second molecular beacon probes are different, and wherein the second molecular beacon probe further comprises a ~~second~~ stem and a loop, wherein the stem comprises ~~comprising~~ about 4 base pairs having a sequence 5'-CACG-3', and ~~a second~~ the loop structure comprising ~~comprises~~ about 19-20 base pairs and a T_m of about 54-56°C; and

detecting the first and second molecular beacon probes hybridized to the target nucleic acid, thereby detecting the target nucleic acid.

112. (Currently Amended) A method for detecting a target nucleic acid in a population of non-target nucleic acid contained in a sample, the method comprising:

dividing a heterogenous sample comprising target nucleic acid and non-target nucleic acid to form a plurality of assay samples, wherein the concentration of non-target nucleic acid is at least 5 fold that of target nucleic acid in the heterogeneous sample, and wherein at least one of the assay samples comprises a single molecule of the target nucleic acid;

amplifying the single molecule of target nucleic acid to form an amplification product;

hybridizing the amplification products to a first molecular beacon probe which hybridizes to the target nucleic acid and a second molecular beacon probe which hybridizes to the non-target nucleic acid, wherein each of the first and the second molecular probes comprises a photoluminescent dye and a quenching agent at opposite 5' and 3' ends,

wherein the photoluminescent dye on the first and second molecular beacon probes are different, and wherein the second molecular beacon probe further comprises a ~~second~~ stem and a loop, wherein the stem comprises ~~comprising~~ about 4 base pairs having a sequence 5'-CACG-3', and a ~~second~~ the loop ~~structure comprising~~ comprises about 19-20 base pairs and a T_m of about 54-56°C; and

detecting the first and second molecular beacon probes hybridized to the target nucleic acid, thereby detecting the target nucleic acid.

113. (Previously Presented) The method of claim 109 wherein the sample is diluted so that between about 0.1 and about 0.9 of the assay samples yield an amplification product when amplified.

114. (Previously Presented) The method of claim 113 wherein the sample is diluted so that between about 0.1 and about 0.6 of the assay samples yield an amplification product when amplified.

115. (Currently Amended) The method of claim 65, 66, or 67, wherein the ~~first~~ loop consists of 16 base pairs and has a T_m of 50-51°C, and wherein the ~~first~~ stem consists of 4 base pairs having a sequence 5'-CACG-3'.

116. (Currently Amended) The method of claim 110, 111, or 112, wherein the ~~second~~ loop consists of 19-20 base pairs and has a T_m of 54-56°C, and wherein the ~~second~~ stem consists of 4 base pairs having a sequence 5'-CACG-3'.

117. (New) The method of claim 66 or 111, wherein X is less than 20.

118. (New) The method of claim 65, 66, 67, 110, 111, or 112, wherein the target nucleic

acid is a mutant nucleic acid.

119. (New) The method of claim 65, 67, 110, or 112, wherein the non-target nucleic acid is a wild-type nucleic acid.

120. (New) The method of claim 65, 66, 67, 110, 111, or 112, wherein one out of every two assay samples contains on average one target nucleic acid.

121. (New) The method of claim 65, 66, 67, 110, 111, or 112, wherein the target nucleic acid is a genomic DNA.

122. (New) The method of claim 65, 66, 67, 110, 111, or 112 wherein the target nucleic acid is a cDNA.

123. (New) The method of claim 122, wherein the cDNA is generated by RT-PCR of at least one RNA.

124. (New) The method of claim 65 or 110, wherein at least ten assay samples are analyzed.

125. (New) The method of claim 124, wherein at least 15 assay samples are analyzed.

126. (New) The method of claim 124, wherein at least 20 assay samples are analyzed.

127. (New) The method of claim 124, wherein at least 25 assay samples are analyzed.

128. (New) The method of claim 124, wherein at least 30 assay samples are analyzed.

129. (New) The method of claim 124, wherein at least 40 assay samples are analyzed.

130. (New) The method of claim 124, wherein at least 50 assay samples are analyzed.

131. (New) The method of claim 124, wherein at least 75 assay samples are analyzed.

132. (New) The method of claim 124, wherein at least 100 assay samples are analyzed.

133. (New) The method of claim 124, wherein at least 500 assay samples are analyzed.
134. (New) The method of claim 124, wherein at least 1000 assay samples are analyzed.
135. (New) The method of claim 65, 66, 67, 110, 111, or 112, wherein the source of the sample is selected from the group consisting of stool, blood and lymph nodes.
136. (New) The method of claim 65, 66, 67, 110, 111, or 112, wherein the amplifying step employs a polymerase chain reaction.
- 137 (New) The method of claim 65, 66, 67, 110, 111, or 112, wherein the first probe detects a wild-type nucleic acid better than a mutant nucleic acid.
138. (New) The method of claim 65, 66, 67, 110, 111, or 112, wherein the first probe detects a mutant nucleic acid better than a wild-type nucleic acid.
139. (New) The method of claim 65, 66, 67, 110, 111, or 112, further comprising an asymmetric amplification step comprising adding a single internal primer and performing amplification.
140. (New) The method of claim 65, 66, 67, 110, 111, or 112, wherein the target nucleic acid comprises one or more mutations selected from the group consisting of base substitutions, chromosomal translocations, gene amplifications and alternatively spliced RNAs.
141. (New) The method of claim 65, 66, 67, 110, 111, or 112, wherein said method detects allelic discrimination, allelic imbalance, amplicons that are amplified during neoplastic development or rare exons.
142. (New) The method of claim 65, 66, 67, 110, 111, or 112, wherein said method detects a change in gene expression or a change in relative expression of a wild-type nucleic acid or

a mutant nucleic acid.

143. (New) The method of claim 110, 111, or 112, wherein the first molecular beacon probe comprises a stem and a loop, wherein the loop comprises about 16 base pairs and has a T_m of about 50-51°C, and wherein the stem comprises about 4 base pairs having a sequence 5'-CACG-3'.

144. (New) The method of claim 143, wherein the loop of the first molecular beacon probe consists of 16 base pairs and has a T_m of 50-51°C, and wherein the stem of the first molecular beacon probe consists of 4 base pairs having a sequence 5'-CACG-3'.

145. (New) The method of claim 67, 111, or 112 wherein said dividing step comprises diluting said sample.

146. (New) The method of claim 145 wherein the sample is diluted so that between about 0.1 and about 0.9 of the assay samples yield an amplification product when amplified.

147. (New) The method of claim 146, wherein the sample is diluted so that between about 0.1 and about 0.6 of the assay samples yield an amplification product when amplified.

148. (New) The method of claim 65, 66, 67, 110, 111, or 112, wherein the target nucleic acid comprises one or more somatic mutation.

149. (New) The method of claim 65 or 110 wherein the separating step comprises diluting said sample.